HUMAN INTERLEUKIN-11 RECEPTOR

Field of the Invention

The present invention relates to the human interleukin-11 receptor, fragments thereof and recombinant polynucleotides and cells useful for expressing such proteins.

Background of the Invention

A variety of regulatory molecules, known as cytokines, have been identified including interleukin-11 (IL-11). The various protein forms of IL-11 and DNA encoding various forms of IL-11 activity are described in Bennett, *et al.*, USPN 5,215,895 (June 1, 1993); McCoy, *et al.*, USPN 5,270,181 (December 14, 1993); and McCoy, *et al.*, USPN 5,292,646 (March 8, 1994), all incorporated herein by reference. Thus, the term "IL-11" includes proteins having the biological activity described in these patents, whether produced by recombinant genetic engineering techniques; purified from cell sources producing the factor naturally or upon induction with other factors; or synthesized by chemical techniques; or a combination of the foregoing.

IL-11 is a pleiotropic cytokine that has been implicated in production of several biological activities including: induction of multipotential hematopoietic progenitor cell proliferation (Musashi et al. (1991) Blood, 78, 1448-1451); enhancement of megakaryocyte and platelet formation (Burstein et al. (1992) J. Cell. Physiol., 153, 305-312); stimulation of acute phase protein synthesis

(Baumann et al. (1991) J. Biol. Chem., 266, 20424-20427); inhibition of adipocyte lipoprotein lipase activity (Kawashima et al. (1991) FEBS Lett., 283, 199-202); and effects on neurotransmitter phenotype (Fann et al. (1994) Proc. Natl. Acad. Sci. USA, 91, 43-47).

IL-11 may be used in a pharmaceutical preparation or formulation to treat immune deficiencies, specifically deficiencies in hematopoietic progenitor cells, or disorders relating thereto. Treatment of the other disorders or stimulation of the immune systems of cells thereof may also employ IL-11. IL-11 may also be employed in methods for treating cancer and other disease. Such pathological states may result from disease, exposure to radiation or drugs, and include, for example, leukopenia, bacterial and viral infections, anemia, B cell or T cell deficiencies such as immune cell or hematopoietic cell deficiency following a bone marrow transplantation. IL-11 may also be used to potentiate the immune response to a variety of vaccines creating longer lasting and more effective immunity. Therapeutic treatment of cancer and other diseases with IL-11 may avoid undesirable side effects caused by treatment with presently available drugs.

Like most cytokines, IL-11 exhibits certain biological activities by interacting with an IL-11 receptor (IL-11R) on the surface of target cells. It would be desirable to identify and clone the sequence for the human receptor so that IL-11R proteins can be produced for various reasons, including production of therapeutics and screening for inhibitors of IL-11 binding to the receptor and receptor signalling.

Summary of the Invention

In accordance with the present invention, polynucleotides encoding the human interleukin-11 receptor are disclosed. In certain embodiments, the invention provides an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 803 to nucleotide 1999;

(b) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) as a result of degeneracy of the genetic code;

(c) an allelic variant of the nucleotide sequence specified in (a); and

(d) a fragment of (a) or (b) encoding a protein having the ability to bind IL-11.

Preferably, the nucleotide sequence encodes a protein having a biological activity of the human IL-11 receptor. The nucleotide sequence may be operably linked to an expression control sequence. In preferred embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO:1 from nucleotide 803 to nucleotide 1999 or a fragment thereof; the nucleotide sequence of SEQ ID NO:1 from nucleotide 803 to nucleotide 1828 or a fragment thereof; the nucleotide sequence of SEQ ID NO:1 from nucleotide 1904 to nucleotide 1999 or a fragment thereof; the nucleotide sequence of SEQ ID NO:1 from nucleotide 734 to nucleotide 1999 or a fragment thereof; the nucleotide sequence of SEQ ID NO:1 from nucleotide 1067 to nucleotide 1828 or a fragment thereof; or the nucleotide sequence of SEQ ID NO:1 from nucleotide 1067 to nucleotide 1999 or a fragment thereof. Other preferred embodments include the nucleotide sequence of SEQ ID NO:1 from nucleotide 734 to nucleotide 1828; the nucleotide sequence of SEQ ID

NO:1 from nucleotide 734 to nucleotide 1810; the nucleotide sequence of SEQ ID NO:1 from nucleotide 734 to nucleotide 1768; and the nucleotide sequence of SEQ ID NO:1 from nucleotide 734 to nucleotide 1705. In other embodiments, the polynucleotide comprises a nucleotide sequence capable of hybridizing to the nucleotides sequence of SEQ ID NO:1 under highly stringent conditions.

The invention also provides isolated polynucleotides comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 422;

(c) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 365;

(d) the amino acid sequence of SEQ ID NO:2 from amino acids 391 to 422;

(e) the amino acid sequence of SEQ ID NO:2 from amino acids 112 to 422;

(f) the amino acid sequence of SEQ ID NO:2 from amino acids 112 to 365; and

(g) fragments of (a)-(f) having a biological activity of the human IL-11 receptor.

Host cells, preferably mammalian cells, transformed with the polynucleotides are also provided.

In other embodiments, the invention provides a process for producing a human IL-11R protein. The process comprises:

(a) growing a culture of the host cell of the present invention in a suitable culture medium; and

(b) purifying the human IL-11R protein from the culture.

Proteins produced according to these methods are also provided.

The present invention also provides for an isolated human IL-11R protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 422;

(c) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 365;

(d) the amino acid sequence of SEQ ID NO:2 from amino acids 391 to 422;

(e) the amino acid sequence of SEQ ID NO:2 from amino acids 112 to 422;

(f) the amino acid sequence of SEQ ID NO:2 from amino acids 112 to 365;

(g) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 359;

(h) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 345;

(i) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 324; and

(j) fragments of (a)-(i) having a biological activity of the human IL-11 receptor. Preferably the protein comprises the amino acid sequence of SEQ ID NO:2; the sequence from amino acid 24 to 422 of SEQ ID NO:2; the sequence from amino acid 24 to 365 of SEQ ID NO:2; or the sequence from amino acid 391 to 422 of SEQ ID NO:2. In other preferred embodiments the protein comprises an amino acid sequence beginning with amino acid 23 or amino acid 26 of SEQ ID NO:2. Pharmaceutical compositions comprising a protein of the present invention and a pharmaceutically acceptable carrier are also provided.

The present invention further provides for compositions comprising an antibody which specifically reacts with a protein of the present invention.

Methods of identifying an inhibitor of IL-11 binding to the human IL-11 receptor are also provided. These methods comprise:

(a) combining a human IL-11R protein or a fragment thereof with IL-11 or a fragment thereof, said combination forming a first binding mixture;

(b) measuring the amount of binding between the protein and the IL-11 or fragment in the first binding mixture;

(c) combining a compound with the protein and the IL-11 or fragment to form a second binding mixture;

(d) measuring the amount of binding in the second binding mixture; and

(e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture;

wherein the compound is capable of inhibiting IL-11 binding to the human IL-11 receptor when a decrease in the amount of binding of the second binding mixture occurs. Optionally, the first and/or second binding mixture may further comprise gp130 or a fragment thereof capable of binding to the protein of claim 11 or the IL-11 or fragment used therein. Inhibitors of IL-11R identified by these methods and pharmaceutical compositions containing them are also provided.

Methods of inhibiting binding of IL-11 to the human IL-11 receptor in a mammalian subject are also disclosed which comprise administering a therapeutically effective amount of a composition containing a human IL-11R protein, an IL-11R inhibitor or an antibody to a human IL-11R protein. Methods of treating or preventing loss of bone mass in a mammalian subject using these compositions are also provided.

In yet other embodiments the invention provides for an isolated polynucleotide comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 422;

(c) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 365;

(d) the amino acid sequence of SEQ ID NO:2 from amino acids 391 to 422;

(e) the amino acid sequence of SEQ ID NO:2 from amino acids 112 to 422;

(f) the amino acid sequence of SEQ ID NO:2 from amino acids 112 to 365;

(g) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 359;

(h) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 345;

(i) the amino acid sequence of SEQ ID NO:2 from amino acids 24 to 324; and

(j) fragments of (a)-(i) having a biological activity of the human IL-11 receptor.

Brief Description of the Figures

Figure 1 depicts a schematic representation of the structures of the human IL-11 receptor and gp130.

Figure 2 presents data demonstrating the biological activity of a soluble form of recombinant human IL-11R protein.

Figure 3 presents data demonstrating the ability of soluble human IL-11R to mediate cell proliferation in the presence of IL-11.

Figure 4 presents data demonstrating the specific binding of soluble human IL-R protein to IL-11.

Detailed Description of Preferred Embodiments

The inventors of the present application have for the first time identified and provided a polynucleotide encoding the human IL-11 receptor (human IL-11R).

SEQ ID NO:1 provides the nucleotide sequence of a cDNA encoding the human IL-11R. SEQ ID NO:2 provides the amino acid sequence of the receptor, included a putative signal sequence from amino acids 1-23. The mature human IL-11R is believed to have the sequence of amino acids 23-422, 24-422 or 26-422 of SEQ ID NO:2. Applicants have found that upon expression human IL-11R proteins are found to have N-termini at either amino acid 23 (approximately 20%), 24 (approximately 24%) or 26 (approximately 50%). References herein to the "mature" form of the protein are intended to refer to forms beginning with any of these amino acids, and are sometimes references herein as having the sequence "23/24/26-422" of SEQ ID NO:2.

The mature receptor has at least three distinct domains: an extracellular domain (comprising approximately amino acids 23/24/26-365 of SEQ ID NO:2), a transmembrane domain (comprising approximately amino acids 366-390 of SEQ ID NO:2) and an intracellular domain (comprising approximately amino acids 391-422 of SEQ ID NO:2). The extracellular domain is further divided into an immunoglobulin-like domain (comprising approximately amino acids 23/24/26-111 of SEQ ID NO:2) and a type-I-cytokine domain (comprising approximately amino acids 112-365 of SEQ ID NO:2).

Soluble forms of human IL-11R protein can also be produced. Such soluble forms include without limitation proteins comprising amino acids 1-365 and 23/24/26-365 of SEQ ID NO:2. The soluble forms of the human IL-11R are further characterized by being soluble in aqueous solution, preferably at room temperature. Human IL-11R proteins comprising only the intracellular domain or a portion thereof may also be produced. Any forms of human IL-11R of less than

full length are encompassed within the present invention and are referred to herein collectively as "human IL-11R" or "human IL-11R proteins." Human IL-11R proteins of less than full length may be produced by expressing a corresponding fragment of the polynucleotide encoding the full-length human IL-11R protein (SEQ ID NO:1). These corresponding polynucleotide fragments are also part of the present invention. Modified polynucleotides as described above may be made by standard molecular biology techniques, including construction of appropriate desired deletion mutants, site-directed mutagenesis methods or by the polymerase chain reaction using appropriate oligonucleotide primers.

Based upon similarity to the structure of the IL-6 receptor, it is predicted that IL-11R proteins containing only the type-I cytokine domain of the extracellular region of the full length receptor will be capable of binding IL-11 and inducing receptor signalling. As a result, IL-11R proteins comprising amino acids 112 to 365 of SEQ ID NO:2, IL-11R proteins comprising amino acids 112 to 390 of SEQ ID NO:2, and IL-11R proteins comprising amino acids 112 to 422 of SEQ ID NO:2 are provided by the present invention. Polynucleotides encoding such proteins (such as for example a polynucleotide comprising the nucleotides sequence of SEQ ID NO:1 from nucleotide 1067 to nucleotide 1828, a polynucleotide comprising the nucleotides sequence of SEQ ID NO:1 from nucleotide 1067 to nucleotide 1906, and a polynucleotide comprising the nucleotides sequence of SEQ ID NO:1 from nucleotide 1067 to nucleotide 1999, respectively) are also provided by the invention.

For the purposes of the present invention, a protein has "a biological activity of the human IL-11 receptor" if it possess one or more of the following

characteristics: (1) the ability to bind IL-11 or a fragment thereof (preferably a biologically active fragment thereof); (2) the ability to bind to cytosolic proteins or molecules involved in the signalling pathway invoked by IL-11 binding to human IL-11R; (3) the ability to produce a signal characteristic of the binding of IL-11 to human IL-11R (where the protein in question either contains a portion able to bind IL-11 or where the protein in question would produce such signal if joined to another protein which is able to bind IL-11); (4) the ability to bind to gp130 or a fragment thereof (either in the presence or absence of IL-11); (5) the ability to induce tyrosine phosphorylation of gp130; (6) the ability to induce tyrosine phosphorylation of JAK kinases; or (7) the ability to induce tyrosine phosphorylation of the STAT family of DNA binding proteins (Zhong et al. (1994) Science 264, 95-98). Preferably, the biological activity possessed by the protein is the ability to bind IL-11 or a fragment hereof, more prefereably with a K_D of about 0.1 to about 100 nM, most preferably with a K_D of about 1 to about 10 nM.

Human IL-11R or active fragments thereof (human IL-11R proteins) may be fused to carrier molecules such as immunoglobulins. For example, soluble forms of the human IL-11R may be fused through "linker" sequences to the Fc portion of an immunoglobulin. Other fusions proteins, such as those with GST, Lex-A or MBP, may also be used.

The invention also encompasses allelic variations of the nucleotide sequence as set forth in SEQ ID NO:1, that is, naturally-occurring alternative forms of the isolated polynucleotide of SEQ ID NO: 1 which also encode human IL-11R proteins, preferably those proteins having a biological activity of human IL-11R. Also included in the invention are isolated polynucleotides which hybridize to the

nucleotide sequence set forth in SEQ ID NO:1 under highly stringent conditions (for example, 0.1xSSC at 65°C). Isolated polynucleotides which encode human IL-11R proteins but which differ from the nucleotide sequence set forth in SEQ ID NO:1 by virtue of the degeneracy of the genetic code are also encompassed by the present invention. Variations in the nucleotide sequence as set forth in SEQ ID NO:1 which are caused by point mutations or by induced modifications are also included in the invention.

The isolated polynucleotides of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the human IL-11R protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means enzymatically or chemically ligated to form a covalent bond between the isolated polynucleotide of the invention and the expression control sequence, in such a way that the human IL-11R protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the human IL-11R protein. Any cell type capable of expressing functional human IL-11R protein may be used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other

transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D, FDCP-1, PC12, M1x or C2C12 cells.

The human IL-11R protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. Soluble forms of the human IL-11R protein may also be produced in insect cells using appropriate isolated polynucleotides as described above.

Alternatively, the human IL-11R protein may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, *Candida,* or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include *Escherichia coli, Bacillus subtilis, Salmonella typhimurium,* or any bacterial strain capable of expressing heterologous proteins.

Expression in bacteria may result in formation of inclusion bodies incorporating the recombinant protein. Thus, refolding of the recombinant protein may be required in order to produce active or more active material. Several methods for obtaining correctly folded heterologous proteins from bacterial inclusion bodies are known in the art. These methods generally involve

solubilizing the protein from the inclusion bodies, then denaturing the protein completely using a chaotropic agent. When cysteine residues are present in the primary amino acid sequence of the protein, it is often necessary to accomplish the refolding in an environment which allows correct formation of disulfide bonds (a redox system). General methods of refolding are disclosed in Kohno, Meth. Enzym., 185:187-195 (1990). EP 0433225 and copending application USSN 08/163,877 describe other appropriate methods.

The human IL-11R protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a polynucleotide sequence encoding the human IL-11R protein.

The human IL-11R protein of the invention may be prepared by growing a culture transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of the human IL-11R protein of the invention can be purified from conditioned media. Membrane-bound forms of human IL-11R protein of the invention can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100.

The human IL-11R protein can be purified using methods known to those skilled in the art. For example, the human IL-11R protein of the invention can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as

a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) or polyetheyleneimine (PEI) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., S-Sepharose® columns). The purification of the human IL-11R protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the human IL-11R protein. Affinity columns including IL-11 or fragments thereof or including antibodies to the IL-11R protein can also be used in purification in accordance with known methods. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein. Preferably, the isolated human IL-11R protein is purified so that it is substantially free of other mammalian proteins.

Human IL-11R proteins of the invention may also be used to screen for agents which are capable of binding to human IL-11R or interfere with the binding of IL-11 to the human IL-11R (either the extracellular or intracellular domains) and

thus may act as inhibitors of normal binding and cytokine action (IL-11R inhibitors). Binding assays using a desired binding protein, immobilized or not, are well known in the art and may be used for this purpose using the human IL-11R protein of the invention. Purified cell based or protein based (cell free) screening assays may be used to identify such agents. For example, human IL-11R protein may be immobilized in purified form on a carrier and binding to purified human IL-11R protein may be measured in the presence and in the absence of potential inhibiting agents. A suitable binding assay may alternatively employ a soluble form of human IL-11R of the invention.

In such a screening assay, a first binding mixture is formed by combining IL-11 or a fragment thereof and human IL-11R protein, and the amount of binding in the first binding mixture (B_o) is measured. A second binding mixture is also formed by combining IL-11 or a fragment thereof, human IL-11R protein, and the compound or agent to be screened, and the amount of binding in the second binding mixture (B) is measured. The amounts of binding in the first and second binding mixtures are compared, for example, by performing a calculation of the ratio B/B_o. A compound or agent is considered to be capable of inhibiting binding if a decrease in binding in the second binding mixture as compared to the first binding mixture is observed. Optionally, gp130 can be added to one or both of the binding mixtures. The formulation and optimization of binding mixtures is within the level of skill in the art, such binding mixtures may also contain buffers and salts necessary to enhance or to optimize binding, and additional control assays may be included in the screening assay of the invention.

Compounds found to reduce the binding activity of human IL-11R protein to IL-11 or its fragment to any degree, preferably by at least about 10%, more preferably greater than about 50% or more, may thus be identified and then secondarily screened in other binding assays and in vivo assays. By these means compounds having inhibitory activity for IL-11R binding which may be suitable as therapeutic agents may be identified.

Human IL-11R proteins, and polynucleotides encoding them, may also be used as diagnostic agents for detecting the expression or presence of IL-11R, IL-11 or cells expressing IL-11R or IL-11. The proteins or polynucleotides may be employed for such purpose in standard procedures for diagnostics assays using these types of materials. Suitable methods are well known to those skilled in the art.

Human IL-11R acts as a mediator of the known biological activities of IL-11. As a result, isolated human IL-11R protein and IL-11R inhibitors may be useful in treatment or modulation of various medical conditions in which IL-11 is implicated or which are effected by the activity (or lack thereof) of IL-11 (collectively "IL-11-related conditions"). IL-11-related conditions include without limitation immune deficiencies, specifically deficiencies in hematopoietic progenitor cells, or disorders relating thereto, cancer and other disease. Such pathological states may result from disease, exposure to radiation or drugs, and include, for example, leukopenia, bacterial and viral infections, anemia, B cell or T cell deficiencies such as immune cell or hematopoietic cell deficiency following a bone marrow transplantation.

It is also believed that IL-11 and IL-11R may play a role in the regulation of bone maturation and repair (Girasole et al. (1994) J. Clin. Invest. 93, 1516-1524; Passeri et al. (1992) J. Bone Miner. Res., 7(S1), S110 Abst.; Passeri et al. (1993) J. Bone Miner. Res., 8(S1), S162 Abst.). As a result, human IL-11R protein and IL-11R inhibitors may be useful in treatment of bone loss (including that associated with osteoporosis, post-menopausal osteoporosis, senile osteoporosis, idiopathic osteoporosis, Pagets disease, multipe myeloma, and hypogonadal conditions).

Human IL-11R protein and IL-11R inhibitors, purified from cells or recombinantly produced, may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to human IL-11R or ligand and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration.

The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, G-CSF, stem cell factor, and erythropoietin. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other anti-inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with isolated human IL-11R protein or IL-11R inhibitor, or to minimize side effects caused by the isolated

human IL-11R or IL-11R inhibitor. Conversely, isolated human IL-11R or IL-11R inhibitor may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which isolated human IL-11R protein or IL-11R inhibitor is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., amelioration of symptoms of, healing of, or increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined

amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of isolated human IL-11R protein or IL-11R inhibitor is administered to a mammal. Isolated human IL-11R protein or IL-11R inhibitor may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, human IL-11R protein or IL-11R inhibitor may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering human IL-11R protein or IL-11R inhibitor in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of human IL-11R protein or IL-11R inhibitor used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of human IL-11R protein or IL-11R inhibitor is administered orally, human IL-11R protein or IL-11R inhibitor will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally

contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% human IL-11R protein or IL-11R inhibitor, and preferably from about 25 to 90% human IL-11R protein or IL-11R inhibitor. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of human IL-11R protein or IL-11R inhibitor, and preferably from about 1 to 50% human IL-11R protein or IL-11R inhibitor.

When a therapeutically effective amount of human IL-11R protein or IL-11R inhibitor is administered by intravenous, cutaneous or subcutaneous injection, human IL-11R protein or IL-11R inhibitor will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to human IL-11R protein or IL-11R inhibitor an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain

stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

The amount of human IL-11R protein or IL-11R inhibitor in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of human IL-11R protein or IL-11R inhibitor with which to treat each individual patient. Initially, the attending physician will administer low doses of human IL-11R protein or IL-11R inhibitor and observe the patient's response. Larger doses of human IL-11R protein or IL-11R inhibitor may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 μg to about 100 mg of human IL-11R protein or IL-11R inhibitor per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the human IL-11R protein or IL-11R inhibitor will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Human IL-11R proteins of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the human IL-11R protein and which may inhibit binding of IL-11 or fragments thereof to the receptor. Such antibodies may be obtained using the entire human IL-11R as an immunogen, or by using fragments of human IL-11R, such as the soluble mature human IL-11R. Smaller fragments of the human IL-11R may also be used to immunize animals. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J.Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Neutralizing or non-neutralizing antibodies (preferably monoclonal antibodies) binding to human IL-11R protein may also be useful therapeutics for certain tumors and also in the treatment of conditions described above. These neutralizing monoclonal antibodies may be capable of blocking IL-11 binding to the human IL-11R.

Example 1

Isolation of Human IL-11R cDNA

Generation of DNA Probes:

DNA probes derived from the murine Etl-2 sequence (SEQ ID NO:3) were obtained by PCR from murine placenta cDNA. The amino terminal probe

corresponds to base pairs 418-570 and the carboxy terminal probe to base pairs 847- 1038 of the murine Etl-2 sequence. The DNA probes were gel purified and radiolabeled using α32P-dATP and α32P-dCTP.

cDNA Library Screening:

cDNA was generated from activated human PBMC using the Superscript Choice System and cloned into the EcoR1 site of ZAP II (Stratagene). The resulting phage were used to infect E. coli strain BB4. One million phage were plated on 150 mm NZCYM plates at a density of 15000 pfu/plate. Plaques were transferred to duplicate Duralose nitrocellulose filters (Stratagene). Following alkali denaturation and heat fixation the filters were pre-hybridized in 5X SSC, 5X Denhardts, 0.1% SDS, and 50 µg/ml yeast tRNA for 2 hours at 65°C. One set of filters was hybridized with the amino-terminal probe and the other set with the carboxy-terminal probe (5 x 10^5 cpm/ml) for 48 hrs at 55°C in pre-hybridization buffer. The filters were washed with 4X SSC, 0.1% SDS once at 25°C and twice at 55°C. Plaques that hybridized to both probes were identified by autoradiography.

Of the one million plaques screened two plaques hybridized to both of the probes. These plaques were picked and the phage eluted into SM media containing chloroform. The resulting phage were used to reinfect E. coli strain BB4 and plated on NZCYM plates at a density of 100-300 pfu/plate for a secondary screen.

Following the secondary screen plasmid DNA was isolated from the ZAPII plaques by excision using helper phage (Stratagene). The DNA sequence of the inserts was determined on an Applied Biosystems DNA sequencer.

Clone phIL11R14-2 containing the polynucleotide having the sequence of SEQ ID NO:1 was deposited with ATCC at accession number 69731 on December 22, 1994.

Example 2

Expression of Soluble Human IL-11R Protein and Assay of Activity

A soluble form of human IL-11R protein was expressed in mammalian cells. The expresssed recombinant protein was capable of transducing a signal in BAF130-9 cells.

A portion of the full length human IL-11R sequence (nucleotides 734-1828 of SEQ ID NO:1 encoding amino acids 1-365 of SEQ ID NO:2) corresponding to a soluble form was cloned into the mammalian expression vector pED and used to transfect COSM6 cells. 40 hours after transfection conditioned media was removed, concentrated 5 fold and used in proliferation assays with the murine cell line BAF130-9 (Hibi, M. et al. (1990) Cell 63, 1149-57), a derivative of the BAFB03 cell line expressing the human gp130 signal transducer. BAF130-9 cells do not proliferate in response to IL-11 or IL-6 alone, but do proliferate in response to a combination of IL-6 and soluble IL-6R (Hibi et al., supra). BAF130-9 cells ($1x10^4$ cell in 0.1ml) were cultured in RPMI 1640 medium/10% FCS with increasing concentrations of recombinant human IL-11 in the absence or presence of 10 µl of conditioned media from mock transfected cells or cells transfected with the soluble human IL-11R sequence. After forty hours the cells were pulse-labeled with ^{3}H-thymidine (0.5 µCi/well) for eight hours and incorporated nucleotide was

determined. As shown in Figure 2, BAF130-9 cells do not proliferate in response to IL-11 or soluble IL-11R alone, but do proliferate in the presence of both IL-11 and soluble IL-11R.

Other human IL-11R proteins can be tested in this model to determine whether they exhibit a "biological activity" of human IL-11R as defined herein.

Example 3

Other Systems for Determination Biological Activity of Human IL-11R Protein

Other systems can be used to determine whether a specific human IL-11R protein exhibits a "biological activity" of human IL-11R as defined herein. The following are examples of such systems.

Assays for IL-11 Binding

The ability of a human IL-11R protein to bind IL-11 or a fragment thereof can be determine by any sutiable assays which can detect such binding. Some suitable examples follow.

Binding of IL-11 to the extracellular region of the human IL-11R protein will specifically cause a rapid induction of phosphotyrosine on the receptor protein. Assays for ligand binding activity as measured by induction of phosphorylation are described below.

Alternatively, a human IL-11R protein (such as, for example, a soluble form of the extracellular domain) is produced and used to detect IL-11 binding. For example, a DNA construct is prepared in which the extracellular domain (truncated prior, preferably immediately prior, to the predicted transmembrane domain) is

ligated in frame to a cDNA encoding the hinge C_H2 and C_H3 domains of a human immunoglobulin (Ig) $\gamma 1$. This construct is generated in an appropriate expression vector for COS cells, such as pEDΔC or pMT2. The plasmid is transiently transfected into COS cells. The secreted IL-11R-Ig fusion protein is collected in the conditioned medium and purified by protein A chromatography.

The purified IL-11R-Ig fusion protein is used to demonstrate IL-11 binding in a number of applications. IL-11 can be coated onto the surface of an enzyme-linked immunosorbent assay (ELISA) plate, and then additional binding sites blocked with bovine serum albumin or casein using standard ELISA buffers. The IL-11R-Ig fusion protein is then bound to the solid-phase IL-11, and binding is detected with a secondary goat anti-human Ig conjugated to horseradish peroxidase. The activity of specifically bound enzyme can be measured with a colorimetric substrate, such as tetramethyl benzidine and absorbance readings.

IL-11 may also be expressed on the surface of cells, for example by providing a transmembrane domain or glucosyl phosphatidyl inositol (GPI) linkage. Cells expressing the membrane bound IL-11 can be identified using the IL-11R-Ig fusion protein. The soluble IL-11R-Ig fusion is bound to the surface of these cells and detected with goat anti-human Ig conjugated to a fluorochrome, such as fluorescein isothiocyanate and flow cytometry.

Interaction Trap

A yeast genetic selection method, the "interaction trap" [Gyuris et al, Cell 75:791-803, 1993], can be used to determine whether a human IL-11R protein has a biological activity of human IL-11R as defined herein. In this system, the

expression of reporter genes from both LexAop-Leu2 and LexAop-LacZ relies on the interaction between the bait protein, for example in this case a species which interacts with huamn IL-11R, and the prey, for example in this case the human IL-11R protein. Thus, one can measure the strength of the interaction by the level of Leu2 or LacZ expression. The most simple method is to measure the activity of the LacZ encoded protein, β-galactosidase. This activity can be judged by the degree of blueness on the X-Gal containing medium or filter. For the quantitative measurement of β-galactosidase activity, standard assays can be found in "Methods in Yeast Genetics" Cold Spring Harbor, New York, 1990 (by Rose, M.D., Winston, F., and Hieter, P.).

In such methods, if one wishes to determine whether the human IL-11R protein interacts with a particular species (such as, for example, a cystoslic protein which binds to the intracellular domain of the human IL-11R *in vivo*), that species can be used as the "bait" in the interaction trap with the human IL-11R protein to be tested serving as the "prey", or *vice versa*.

CAT Induction System

Transcription of acute phase plasma protein genes, such as the rat β-fibrinogen gene, is activated by IL-11 in a variety of cell lines. In one exemplary system, COSM6 cells are cotransfected with plasmids encoding the human IL-11R protein (such as the full length human IL-11R or a soluble form thereof), the human gp130 signal transducer and a reporter gene containing the 350 base pair promoter region of the rat b-fibrinogen gene fused to a reporter gene, CAT (Baumann et al. (1991) J. Biol. Chem. 266, 20424-27). The cells are stimulated

with increasing concentrations of recombinant human IL-11 and transcription of the reporter gene is monitored by assaying for the presence of CAT activity.

Phosphorylation of gp130

Activity may also be determined by examining the ability of IL-11 to induce tyrosine phosphorylation of gp130 in cells transfected with a sequence encoding the human IL-11R protein (such as the full length human IL-11R or a soluble form thereof) (Luttcken et al. (1994) Science 263, 89-92).

Phosphorylation of STATs

Activity may also be determined by examining the ability of IL-11 to induce tyrosine phosphorylation of STATs (*s*ignal *t*ransducers and *a*ctivators of *t*ranscription, a family of DNA binding proteins) in cells transfected with a sequence encoding the human IL-11R protein (such as the full length human IL-11R or a soluble form thereof) (Zhong et al. (1994) Science 264, 95-98).

Phosphorylation of JAK Kinases

Activity may also be determined by examining the ability of IL-11 to induce tyrosine phosphorylation of JAK kinases in cells transfected with a sequence encoding the human IL-11R protein (such as the full length human IL-11R or a soluble form thereof) (Yin et al. (1993) J. Immunol. 151: 2555-61).

Example 4
Preparation of Soluble Forms

Soluble forms of the human IL-11R proteins were expressed in mammalian cells. The expressed recombinant proteins were capable of transducing a signal in BAF103-9 cells.

Portions of the full length human IL-11R sequence (nucleotides 734-1828 of SEQ ID NO:1 encoding amino acids 1-365 of SEQ ID NO:2; nucleotides 734-1810 of SEQ ID NO:1 encoding amino acids 1-359 of SEQ ID NO:2; nucleotides 734-1768 of SEQ ID NO:1 encoding amino acids 1-345 of SEQ ID NO:2; nucleotides 734-1705 of SEQ ID NO:1 encoding amino acids 1-324 of SEQ ID NO:2) corresponding to soluble forms were cloned into the mammalian expression vector pED an used to transfect COSM6 cells. It is believed that these constructs produced mature proteins beginning with amino acid 23, 24 or 26 of SEQ ID NO:2 and ending with the specified amino acid.

40 hours after transfection conditioned media was removed, concentrated 5 fold and used in proliferation assays with the murine cell line BAF130-9, a derivative of BAFB03 expressing the human gp130 signal transducer. BAF130-9 cells do not proliferate in response to IL11 or IL6, but do proliferate in response to IL6 and soluble IL6R (Hibi, M. et al. (1990) Cell 63, 1149-57). BAF130-9 cells ($1x10^4$ cell in 0.1ml) were cultured in RPMI 1640 medium/10% FCS with increasing concentrations of rhIL-11 in the absence or presence of 10 µl of conditioned media from mock transfected cells or cells transfected with the putative soluble hIL11R. After forty hours the cells were pulse-labeled with ^{3}H-thymidine (0.5 uCi/well) for four hours and incorporated nucleotide determined. As shown in Figure 3 BAF130-9 cells do not proliferate in response to IL-11 or

soluble IL11R alone but do proliferate in the presence of both IL-11 and any of the soluble forms of the human IL11R.

Example 5
IL-11 Binding Assay Involving Soluble Forms

A DNA construct was prepared in which the extracellular domain (corresponding to nucleotides 734-1828 of SEQ ID NO:1 encoding amino acids 1-365 of SEQ ID NO:2 (mature form 23/24/26-365)) was ligated in frame to a cDNA encoding the hinge C_H2 and C_H3 domains of a human immunoglobulin (Ig) γ1. The resulting cDNA was ligated into pED to generate plasmid pED-Fc-IL11R. A CHO line expressing the fusion protein was generated and the secreted IL-11R-Ig fusion protein was collected from the conditioned media and purified to homogeneity by protein A affinity chromatography.

The purified IL-11R-Ig fusion protein was used to demonstrate binding to IL-11 in an enzyme-linked immunosorbant assay (ELISA). Briefly, IL-11 was coated onto the surface of an 96-well ELISA plate, and the additional binding sites blocked with casein. The IL-11R-Ig fusion protein was then added to the plate and specific binding was detected using protein A conjugated to horseradish peroxidase. Binding activity was assayed using a colorimetric substrate (tetramethyl benzidine) and monitoring absorbance at 490 nm. As shown in Figure 4, the IL-11R-Ig fusion protein binds to the IL-11 coated plates, whereas an IgER-a-Ig fusion protein does not. Furthermore, the binding specific since it can be competed if the IL-11R-Ig fusion protein is first preincubated with an excess of IL-11 but not with IL-6.

All patent and literature references cited herein are incorporated by reference as if fully set forth.